Table I. Removal of Cell Rubber by Centrifugation (at 925 × Gravity) of Alkaline Dispersion

Centrifugation Time, Min.	Rubber Content of Liquor, Mg./100 Ml.	Proportion of Total Rubber, %
0	198	(100)
5	49	25
10	50	25
20	45	23
40	34	17

Table II. Number and Weight Distribution of Globules of Cryptostegia Leaf Cell Rubber

Av. Diam. of Globules, μ	% in Whole Liquor B		% in Gravity Cream A		% in Centrifuged Liquor C	
	No.	Wt.	No.	Wt.	No.	Wt.
1	67	4.2	0	0	72	24
2	24	12	10	0.3	28	76
3.5	3.5	9	23	4	0	0
5	2.2	17	25	13		
6.5	1.4	25	24	26		
8	0.8	27	12	26		
9.5	0.1	6.4	1.1	4.2		
11	0	0	2.4	18		
12.5			1.1	8.9		

radii in Stokes equation gave satisfactory agreement with the observed rate of creaming.

Microscopic and chemical data indicate that a maximum of about 80% of the rubber in this sample could be recovered by direct creaming of the suspension. Attempts to find creaming agents or conditions which would facilitate the separation of the smaller particles were not successful.

These data were obtained on globules prepared from a typical sample of Cuban leaves having a rubber content of 3.2%. A sample of mature selected leaves later obtained from the U. S. Rubber Company plantation at Yuma, Ariz., had a rubber content of 6.9%, and a large proportion of the rubber globules were 30 to 40μ in diameter. It is obvious that the rate of creaming depends upon the size of the globules. It has been well established that the rubber content (*13*) and the size of the globules vary with the age of the leaves (*9*, *19*), and therefore the rate of creaming varies with the maturity of the leaves.

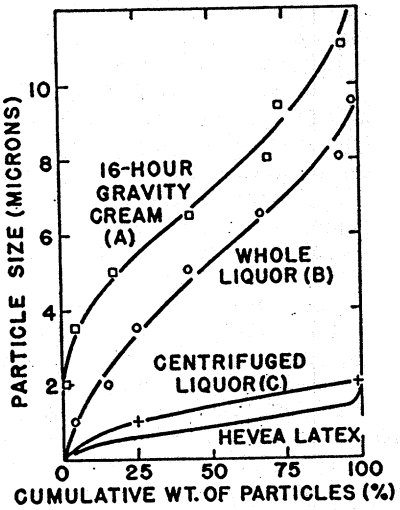

Figure 3. Cumulative Weight Distribution of Cryptostegia Cell Rubber Globules

Composition of Leaf Cell Rubber. Samples of globule rubber were prepared by gravity creaming and centrifuging. The first sample was allowed to cream for 14 days. The cream was then drawn off, diluted with water, and recreamed three times at 48-hour intervals to remove the alkali and other contaminants. The final cream, containing about 50% solids, was dried in vacuo at 50° C. Other samples were creamed for 2 to 4 days, and an antioxidant (JZF) was introduced into the alkaline liquor. The recovery of rubber hydrocarbon by this method ranged from 70 to 75%, which agrees well with the data obtained in the creaming studies. Samples were also prepared by centrifugation to eliminate the effect of long exposure to the air during creaming. The cell rubber was recovered in a total time of less than 3 hours by centrifuging the alkaline cook liquor, adjusting the pH to 4.6, and washing the cream twice. Table III gives analytical data on typical preparations. The crude rubbers obtained both by gravity creaming and by centrifuging were soft and tacky, and contained a large proportion of acetone-soluble material (resins).

Rubber deresinated by precipitation from benzene solutions with acetone was prepared for further studies of its properties. It was firmer than the resiniferous material but still relatively soft and tacky. Inasmuch as the physical properties indicated that it was a relatively low polymer rubber, it was extracted with methyl ethyl ketone (MEK), which is considered to be a solvent for the lower-molecular-weight fraction of hevea and for depolymerized rubber (*5*). The leaf cell rubber was almost completely soluble in MEK (Table IV). Comparative data were obtained upon a sample of smoked sheet of cryptostegia latex rubber which had been exposed to the air for several months. The MEK solubility indicates that the latex rubber is comparable to hevea latex rubber in this respect. Cheyney found that 5 to 8% of crepe and vulcanized rubber and 25% of reclaim were extracted by MEK (*5*).

Further evidence concerning the molecular weight of the cell rubber was obtained by measuring the viscosity of dilute benzene solutions of the rubber. These experiments were based on the methods of Kemp and Peters (*6*). Several samples gave molecular weight values in the range 10,000 to 16,000. These results are consistent with the physical properties and solubility relations, all of which indicate that the leaf cell rubber is of relatively low molecular weight.

The possibility that there had been oxidative breakdown of the rubber during drying, storage, and shipment of the leaves from Cuba was investigated. Several samples of mature leaves were prepared at Yuma by (*a*) air drying and (*b*) sterilizing by canning the fresh leaves in water. It was assumed that oxidative breakdown would be minimized in the sterilized samples. These leaves were shipped to Philadelphia, and globule rubber was prepared from each by the anaerobic fermentation process. There was no significant difference in the acetone-soluble contents (resins) of these two preparations, nor did they differ from results previously obtained upon Cuban leaves (Table IV). It was therefore concluded that no appreciable oxidative breakdown was caused by drying and storing the leaves.

Compounding and Physical Properties. Samples of crude cell rubber prepared by the caustic-creaming process were compounded by a modified A.S.T.M. evaluation procedure. The recipes (Table V) were similar to those employed by McKennon and Lindquist compounding rubber obtained from goldenrod by extraction methods (*10*). Considerable difficulty was encountered in securing satisfactory dispersion of the compounding ingredients in the soft crude stock.

Because of the limited quantities of rubbers available, a test slab 0.030 inch thick was substituted for the usual 0.075- or 0.100-inch slabs. Also, sandwich-type, stainless steel molds having exceptionally broad bands were used because of the excessive plasticity of the compounded stocks. A curing temperature of 260° F. was used for the gum stock recipes, except for certain slow-curing samples which were cured at 274°. The thin test specimens probably had slightly greater tensile strengths than specimens of standard thickness, but the increase would probably

Table III. Composition of Cryptostegia Leaf Cell Globules Obtained by Caustic Creaming Process

Sample	% Rubber Hydrocarbons	% Resins	% Benzene-Insoluble Material
Gravity-creamed	68.3	30.9	0.8
Gravity-creamed	57.5	38.2	4.3
Centrifuged	52.7	45.5	1.8
Centrifuged	62.3	36.2	1.5

Table IV. Composition of Cryptostegia Leaf Cell Rubber[a] and Solubility in MEK

Source of Rubber	Rubber, %	Resins, %	Benzene Insol., %	Sol. in MEK, %
Typical Cuban leaves	57	39	4	93
Dried Yuma leaves	64	33	3	91
Canned Yuma leaves	60	37	3	94
Typical Cuban leaves, pptd. from benzene soln. by 2 vol. acetone	91	9	0	100
Latex, smoked sheet (Haiti)	..	9	..	17

[a] Rubber was determined as tetrabromide, insolubles by direct extraction, and resins by difference (*20*). Solubility in MEK was determined by direct extraction.

TABLE V. RECIPES USED IN EVALUATING CRYPTOSTEGIA LEAF RUBBER STOCKS

	Recipe, Parts		
	A	B	C
Crude cryptostegia rubber	100	100	100
Zinc oxide	6	6	6
Mercaptobenzothiazole (Captax)	1	1	1
Stearic acid	4	4	4
Diphenylguanidine (DPG)	0.5	0.5	0.5
Sulfur	3.5	3.5	2.5
Reinforcing black (Standard Micronex)	...	30	30

TABLE VI. COMPOSITION AND PHYSICAL PROPERTIES OF CRYPTOSTEGIA LEAF CELL RUBBER RECOVERED BY CAUSTIC CREAMING

	Recipe A			Recipe B	
	Resiniferous		Deresinated, creamed	Resiniferous, centrifuged	Deresinated, creamed
	Creamed	Centrifuged			
Composition, %					
Rubber hydrocarbon	68.3	52.7	85.0	52.7	85.0
Resins[a]	30.9	45.5	14.3	45.5	14.3
Benzene-insol. material	0.8	1.8	0.7	1.8	0.7
Physical properties					
Optimum cure, min.	25	20	20	35	20
Cure temp., ° F.	260	274	260	274	274
Optimum tensile, lb./sq. in.	400	1000	1500	650	1950
Ultimate elongation, %	720	630	680	350	550
Modulus at 500%, lb./sq. in.	...	....	600	...	1750
Hardness (Durometer)	25	28	37	57	60

[a] Includes 1% of JZF antioxidant.

not be greater than 200 pounds per square inch. Table VI shows tensile properties of the cryptostegia leaf cell rubber as recovered by caustic creaming.

To determine the effect of resins on the properties of cell rubber, the resiniferous stock was dissolved in benzene and precipitated by acetone. Table VI also shows the composition and physical properties of the deresinated rubber. It is evident that the deresinated cryptostegia leaf cell rubber recovered by the caustic-creaming process was superior in tensile properties to the resiniferous creamed product. Improvement in tensile strength resulting from deresination is probably due in part to improved dispersion of the ingredients as well as to reduction of nonrubber constituents in the specimen.

SOLVENT-EXTRACTION PROCESS

The possibility of direct solvent extraction of the fermentation products appeared worthy of investigation. This method would have the advantage of recovering the latex duct rubber in the leaves as well as the cell rubber not recovered by creaming.

Fermentation as an aid to solvent extraction of rubber was patented in 1873 (*7*). That rubber can be more easily extracted from fermented plant materials has been repeatedly demonstrated (*15*, *17*). Moreover, the removal of nonrubber constituents and the consequent increase in rubber content of the extractor charge decreases the cost of extraction greatly. Improvement in aging properties caused by leaching of metal salts, etc., during fermentation might be an additional advantage. This factor was not investigated, however, as it was impossible to prepare the leaf rubber satisfactorily by any method which did not involve fermentation or leaching.

Table VII gives typical data on the rubber content and bulk of the raw leaves and fermentation products. The increase in rubber content of the extractor charge averaged 2.9 fold on a weight basis and 3.3 fold on a volume basis. Recovery of rubber was essentially quantitative in all four experiments. These fermentations were run for two days, as in the previous work. Unpublished work has shown that greater decomposition can be obtained in additional time; the gain, however, is not great, and the products become progressively more slimy and difficult to filter.

RATE OF EXTRACTION. Ease of extraction of both resins and rubber from the fermented leaves was markedly increased. Data to illustrate this effect were obtained by periodically weighing the extract of small samples extracted by hot percolation of solvent in the regular analytical equipment (Figure 4). The resin extractions were run for 24 hours; the residues were dried in a current of air until free of acetone, and then extracted with benzene. Both extractions proceeded more rapidly in the fermented materials. The difference in the rate of extraction of rubber was especially great. In fact, the rubber could not be completely recovered from untreated leaves even with prolonged extraction[1], whereas extraction of rubber from the fermented leaves was essentially complete in 2 hours under the conditions employed. Lack of pilot-plant equipment made it impossible to obtain data under conditions comparable to those used in large-scale solvent extractors.

METHODS OF EXTRACTION. Solvent extraction can be applied to both protoplasts and bagasse when the two fractions are separated by screening. The bagasse contains the latex duct rubber of the leaves, which presumably is of better quality than the cell rubber. Any protoplast rubber not released by fermentation would also be in this fraction.

Although in practice the fermentation products would probably be recovered and dried without fractionation, for purposes of evaluation the two fractions were prepared by screening the fermented slurry (through 80 mesh) and drying in air at 70° C. The protoplast fraction was ground in a disk mill to pass a 20-mesh screen. The bagasse was cut through a 1/8-inch screen in a rotary knife cutter. The extractions were carried out in an improvised steam-jacketed Soxhlet-type extractor of approximately 0.8 cubic foot capacity. Two types were used: (1) direct extraction with benzene and precipitation of the rubber with 2.5 volumes of acetone, and (2) acetone extraction (deresination) followed by benzene extraction. The rubber obtained by both procedures was freed of solvent and dried in vacuo, after the addition of 1% powdered JZF antioxidant (estimated on the amount of rubber).

LEAF RUBBER FRACTIONS. Table VIII shows the composition and tensile properties of the cell and bagasse rubber fractions as prepared by process 1. The treatment did not completely deresinate either sample, and the bagasse fraction contained more than 20% nonrubber material. In this experiment 35% of the total rubber was in the bagasse portion, an indication that separation of the protoplasts was incomplete and that latex rubber was contaminated with cell rubber. Nevertheless, the crude bagasse rubber was considerably firmer and less tacky than the cell rubber. The tensile properties of the bagasse fraction were definitely better and probably reflected the better quality of the latex duct rubber it contained.

The protoplasts were then extracted by procedure 2, first with acetone and then with benzene. The benzene solution was divided into two portions; one was precipitated with acetone, and the second was evaporated dry in vacuo. Both prepara-

TABLE VII. ENRICHMENT IN CRYPTOSTEGIA LEAF RUBBER BY FERMENTATION BY *Clostridium roseum* AT 40° C. FOR TWO DAYS

Expt. No.	Original Leaves				Fermentation Products				Enrichment Factor for Rubber	
	Dry wt., lb.	Vol., cu. ft.	Rubber Content		Dry wt., lb.	Vol., cu. ft.	Rubber Content		By wt.	By vol.
			%	Lb.			%	Lb.		
S25N30	16.44	0.85	4.2	0.684	6.02	0.27	11.0	0.662	2.6	3.2
S24N3	52.9	2.7	2.8	1.455	18.06	0.82	8.0	1.434	3.1	3.3
S24N4	51.8	2.7	2.5	1.257	17.82	0.81	6.7	1.190	2.7	3.3
S24N5	52.2	2.7	2.2	1.170	17.74	0.84	6.6	1.170	3.0	3.2

[1] Willits and co-workers (*20*) had previously found it necessary to use special chemical treatments prior to extraction to obtain complete extraction of rubber from untreated leaves.

ons of the cell rubber were almost free of resins. Table IX presents the composition and tensile properties of the two preparations compounded by the gum recipe A. The difference between the two samples is slight, and the results agree well with those obtained with the deresinated cell rubber prepared by the creaming method (Table VI). The tensile properties of both samples were superior to those shown by the less completely deresinated sample of cell rubber in Table VIII.

WHOLE LEAF RUBBER. Properties of the mixture of duct and cell rubber which would be obtained if the fermented residues were extracted without fractionation were determined. The small amount of higher-grade duct rubber would certainly not justify extraction for this fraction alone, but there would be no point in discarding it if solvent extraction were to be utilized for recovery of the leaf rubber. For this experiment and the following one on blending with GR-S, several fermentations were carried out (Table VII). The leaf fractions were separated, dried, ground, and extracted with acetone and benzene. [The acetone extract, "resin", investigated by White and Senti (*18*) as a by-product of possible value, contained ursolic acid and higher paraffins typical of leaf and fruit cuticle waxes.] The benzene extracts were freed of solvent in vacuo. Yields ranged from 95 to 105%, on the basis of the rubber content of the leaves fermented. The rubber from the two fractions was combined on a cold mill in the same proportions as the fractions bore to the original amount in the leaves (27% bagasse rubber to 73% cell rubber).

The mixture was so tacky that it was unsuitable for processing according to standard rubber mill techniques. It should be noted here that the solution method of compounding developed by McKennon and Lindquist (*10*) for solvent-extracted goldenrod rubber would be directly applicable to cryptostegia leaf rubber and would make it suitable for processing on conventional rubber mill equipment. However, this type of compounding and precuring was not investigated in this experiment. When this stock was compounded on a roll mill by recipe A and cured in a standard 6 × 6 inch A.S.T.M. test slab mold, it was too soft to be retained in the mold, and the resulting vulcanizate was porous. Presumably it could have been compounded and cured in the smaller quantity and special mold used in the previous work.

TABLE VIII. COMPOSITION AND PROPERTIES OF RUBBER RECOVERED FROM CELL AND BAGASSE FRACTIONS OF CRYPTOSTEGIA LEAF BY SOLVENT EXTRACTION AND ACETONE PRECIPITATION

	Cell Rubber	Bagasse Rubber
Composition, %		
Rubber hydrocarbon	86.4	77.1
Resins[a]	10.7	22.0
Benzene-insol. material	2.9	0.9
Physical properties[b]		
Optimum cure, min. at 260° F.	20	20
Tensile strength, lb./sq. in.	1050	2050
Ultimate elongation, %	730	720
Modulus (600%), lb./sq. in.	150	1000
Hardness (Durometer)	31	40

[a] All resin values include 1% JZF.
[b] Compounded by gum recipe A.

TABLE IX. EFFECT OF RECOVERY BY PRECIPITATION OR EVAPORATION ON COMPOSITION AND PROPERTIES OF SOLVENT-EXTRACTED CELL RUBBER

	Pptn. from Benzene by Acetone	Evapn. of Benzene
Composition, %		
Rubber hydrocarbon	95.3	94.0
Resins	3.8	5.9
Benzene-insol. material	0.9	0.0
Physical properties[a]		
Optimum cure, min. at 260° F.	25	20
Tensile strength, lb./sq. in.	1700	1600
Ultimate elongation, %	660	590
Modulus at 400%, lb./sq. in.	<100	<100
Hardness (Durometer)	43	43

[a] Compounded by gum recipe A.

TABLE X. COMPOSITION AND PROPERTIES OF WHOLE CRYPTOSTEGIA LEAF RUBBER (CONTAINING 27% BAGASSE RUBBER AND 73% CELL RUBBER)

	Bagasse Fraction		Cell Fraction	
Composition, %				
Rubber hydrocarbon	84.8		89.6	
Resins	14.8		10.1	
Benzene-insol. material	0.4		0.3	
Physical properties of blend[a]	Cures			
Curing time, min. at 287° F.	20	35	65	90
Tensile strength, lb./sq. in.	2350	2400	2300[b]	1650
Ultimate elongation, %	560	500	500	400
Modulus, lb./sq. in.				
At 200%	460	480	480	490
At 300%	810	840	900	1000
At 400%	1350	1380	1480	1650
At 500%	1930	2090	2280	..

[a] Compounded by recipe C.
[b] One dumbbell only.

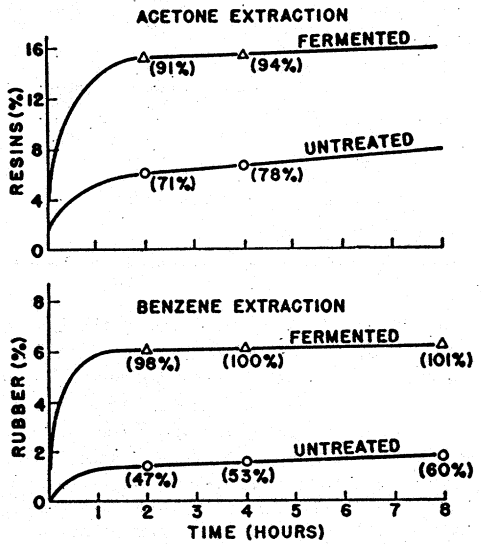

Figure 4. Rate of Extraction of Resins and Rubber from Fermented Cryptostegia

Figures in parentheses show percentage of total amount of constituent extracted.

The stock was therefore compounded by recipe C and cured at 287° F. under standard A.S.T.M. conditions. Table X shows curing characteristics and tensile properties together with the composition.

The curing characteristics and tensile properties of the whole leaf rubber recovered by solvent extraction were, in general, inferior to those of latex rubbers (Hevea, cryptostegia, or koksaghyz). An accelerator-activator (0.5 part diphenylguanidine) was necessary to obtain a satisfactory cure. Although the tensile strength was not high, 2400 pounds per square inch with 500% elongation, the modulus compared favorably with those obtained on latex rubbers with equivalent loading. The cure was reasonably flat, with little or no evidence of reversion caused by overcure.

BLEND OF CRYPTOSTEGIA LEAF RUBBER AND GR-S

The possibility of using these stocks as modifiers for synthetic rubber was also considered. Carlton and Reinbold (*2*) observed that when hevea rubber was combined with GR-S in a ratio of approximately 25 hevea to 75 GR-S, the mixture could be compounded by a GR-S formula without the addition of softeners. This blend had improved processing characteristics, and the tensile properties, resilience, and tear resistance of the vul-

TABLE XI. RECIPES USED IN EVALUATING A BLEND OF CRYPTOSTEGIA LEAF RUBBER AND GR-S

	Blend, Parts	Control, Parts
GR-S	80	100
Cryptostegia	20	...
Zinc oxide	5	5
Softener (Bardol)	5	5
Cyclohexyl benzothiazyl sulfonamide (Santocure)	1	1
Semireinforcing black (Pelletex)	50	50
Sulfur	3	3

TABLE XII. PHYSICAL PROPERTIES OF GR-S BLENDED WITH CRYPTOSTEGIA LEAF RUBBER

Physical Properties	Optimum Cure (20 Min. at 287° F.) Blend[a]	Optimum Cure (20 Min. at 287° F.) Control[b]	Overcure (35 Min. at 287° F.) Blend[a]	Overcure (35 Min. at 287° F.) Control[b]
Green				
Tensile strength, lb./sq. in.	1590	1480	1710	1630
Ultimate elongation, %	450	540	360	330
Modulus, lb./sq. in.				
At 100%	210	120	260	240
At 200%	310	320	790	840
At 300%	990	670	1430	1490
Hardness (Durometer)[c]	52	47	57	60
Tear resistance	..	..	123	101
Aged 100° C. for 96 hours				
Tensile strength, lb./sq. in.	1300	1620	1130	1290
Ultimate elongation, %	170	190	160	150
Modulus, lb./sq. in.				
At 100%	500	460	540	600
At 200%	..	..	..	..
At 300%	..	..	..	..

[a] 20 parts cryptostegia and 80 parts GR-S.
[b] 100 parts GR-S.
[c] Instantaneous reading.

canizate were superior to those of the GR-S control. Morris and co-workers (*11*) expanded these blending studies to other natural rubbers, including cryptostegia. The cryptostegia stock was latex rubber and presumably had physical properties roughly comparable to those of hevea. Since solvent-extracted cryptostegia leaf rubber is low-grade rubber—in comparison with cryptostegia latex rubber, for example—it was of some interest to determine whether the leaf rubber could also be used to improve the physical properties of GR-S stocks.

A compounding recipe (Table XI) similar to the high sulfur recipe of Morris *et al.* was used. The GR-S was given a preliminary breakdown on a cold 6- by 12-inch standard rubber mill, and all the chemicals were added according to the A.S.T.M. procedure, a master batch technique being used. The master batch was divided into two equal parts, and to one the cryptostegia stock (Table X) was added on the basis of 80 parts GR-S to 20 parts cryptostegia; a warm mill (160° F.) was used for blending. A control was provided by adding unmilled GR-S to the remainder of the master batch stock in the same ratio. The plasticity (Williams at 70° C. for 5 minutes) of the blended stock was 0.097 inch; that of the control was 0.110 inch.

The blend of cryptostegia and GR-S stock and the GR-S control were cured at 287° F. in standard A.S.T.M. molds. Limited tensile properties of the vulcanizates are shown in Table XII.

The blending of 20 parts of cryptostegia leaf rubber with 80 parts of GR-S yielded a vulcanizate whose physical properties differed only slightly from those of the control stock. The differences observed could be ascribed to variations in rate of cure. In general, the physical properties of the blend of cryptostegia leaf rubber and GR-S stock paralleled those of a similar stock tested by Morris *et al.*—namely, a blend of goldenrod and GR-S. [Like cryptostegia cell rubber, goldenrod rubber also occurs in the chlorenchyma cells as discrete globules (*14*); chemical data (*16*) indicate that it is similar to the cryptostegia cell rubber.]

Morris and co-workers demonstrated substantial improvement in elongation of GR-S carcass stock by the addition of 20% natural rubber. However, this improvement was not retained after the blended stock had been aged for 96 hours at 100° C. Their data also showed that the tear resistance of the natu rubber and GR-S blends was greater than that of the GR-S co trol.

ECONOMIC CONSIDERATIONS

The primary purpose of the work was accomplished. The two fractions of the leaf rubber were isolated, and their character and properties were fairly well established. The cell rubber was given the major emphasis, for previously little was known about it. A method involving preliminary fermentation was worked out for recovery of the leaf rubber, which should be feasible if rubber of this type is ever required. Economic aspects of the recovery process have not been evaluated because the cost depends primarily upon the rubber content and yield per acre of leaves. Such information should be available upon the completion of agronomic studies now being carried out by the U. S. Department of Agriculture. The fermentation process, which is relatively simple, produces a three-fold increase in the rubber content, regardless of the actual initial rubber content of the leaves. The cost of the solvent-extraction step likewise would depend upon the rubber content of the material. If leaves of high rubber content could be produced cheaply, the over-all cost of the recovery process probably would not be unreasonable in an emergency.

Solvent extraction of the fermentation products recovers all the rubber of the leaf. The properties of this rubber differ little from those of the cell rubber, which makes up the major portion of the total. Although the duct rubber is of higher quality than the cell rubber, preparing it separately does not appear feasible, since it constitutes only 10 to 15% of the total.

SUMMARY

Leaves of *Cryptostegia grandiflora* contain 2 to 7% rubber. The greater part of this rubber (85 to 90%) occurs in discrete globules within the individual chlorenchyma cells; the remainder (10 to 15%) is in the latex vessels of the leaf.

An anaerobic fermentation of the leaves by *Clostridium roseum* for two days at 35° to 40° C. decomposed the cell walls so that the cell content (protoplasts), bearing the globules, was readily separated from the latex vessels in the veins, the cuticle, etc., by screening the fermentation products. The rubber was then recovered from the two fractions by solvent extraction. An alternative procedure for recovery of the cell rubber consisted in alkaline digestion of the protoplasts followed by creaming the rubber globules.

Removal of nonrubber constituents by fermentation effected a 2.9 fold enrichment in rubber content calculated on a weight basis and a 3.2 fold enrichment on a volume basis. In addition, the rate of solvent extraction of both resins by acetone and rubber by benzene was markedly increased.

The cell rubber was a relatively soft, low-polymer rubber, soluble in methyl ethyl ketone, which could be compounded to produce a gum tensile strength of 1500–1700 pounds per square inch, with an ultimate elongation of 600–700%.

The duct rubber of the leaves, which comprised about 15% of the total, was latex rubber of relatively higher quality than the cell rubber but contained all the water-insoluble constituents of the latex.

The rubber of the whole leaf, consisting of the cell rubber and the duct rubber, was prepared by fermentation followed by solvent extraction of the fermentation products. Although the mixture was of slightly better quality than the cell rubber, it wa relatively soft and tacky. When compounded in a carbon bla recipe, it had a tensile strength of 2400 pounds per square inch with an ultimate elongation of 500%. The compounded stock cured to optimum tensile strength in 35 minutes at 274° F.; the curing curve was flat and exhibited no evidence of reversion when the rubber was overcured.

The leaf rubber, when blended with GR-S in the proportions to 80 and compounded by a soft-carcass stock recipe, proced a vulcanizate having substantially the same properties as the GR-S control.

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LITERATURE CITED

(1) Anonymous, *Bull. Imp. Inst.*, 10, 210–12 (1912).
(2) Carlton, C. A., and Reinbold, E. B., *Rubber Age*, 52, 29 (1942).
(3) Cheyney, L. E., IND. ENG. CHEM., 34, 1426–8 (1942).
(4) Dolley, C. S., *India-Rubber J.*, 41, 1155–8 (1912).
(5) Jenkins, D. W., Board of Economic Warfare, *Tech. Bull.* 3 (1943).
(6) Kemp, A. R., and Peters, H., IND. ENG. CHEM., 33, 1263–9 (1941).
(7) Lamb, D. N., U. S. Patent 144,623 (Nov. 18, 1873).
(8) Lucas, F. F., IND. ENG. CHEM., 30, 146 (1938).
(9) McGavack, J., and Hoover, S. R., "Brief Study of Cryptostegia Problem in Haiti", unpub. rept. to Rubber Development Corp., June 15, 1943.
(10) McKennon, F. L., and Lindquist, J. R., *Rubber Age*, 56, 289–92 (1944).
(11) Morris, R. E., Barret, A. E., Harmon, R. E., and Werkenthin, T. A., IND. ENG. CHEM., 36, 60 (1944).
(12) Naghski, J., White, J. W., Jr., Hoover, S. R., and Willaman, J. J., *J. Bact.*, in press.
(13) Polhamus, L. G., Hill, H. H., and Elder, J. A., U. S. Dept. Agr., *Tech. Bull.* 457 (1934).
(14) Rollins, M. E., Bailey, T. L. W., Jr., and deGruy, I. V., *India Rubber World*, in press.
(15) Saunders, W., *Proc. Am. Pharm. Assoc.*, 23, 655–8 (1875).
(16) Skau, E. L., Runckel, W. J., Kreeger, F. B., and Sullivan, M. A., *J. Phys. Chem.*, in press.
(17) Spadaro, J. J., Pollard, E. F., and Porges, N., *Rubber Age*, 56, 53 (1944).
(18) White, J. W., Jr., and Senti, F. R., *J. Am. Chem. Soc.*, 67, 881 (1945).
(19) Whittenberger, R. T., Brice, B. A., and Copley, M. J., *India Rubber World*, 112, 319–23 (1945).
(20) Willits, C. O., *et al.*, in preparation.

CRYPTOSTEGIA LEAF RUBBER

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The leaves of *Cryptostegia grandiflora* have been investigated as a possible emergency source of rubber, supplementing the latex rubber from the stems. The leaf rubber consists of two distinct portions; 85 to 90% is a relatively low-polymer rubber which occurs in the chlorenchyma cells, and 10 to 15% is a latex rubber of better quality which occurs in the latex ducts. Isolation of the leaf rubber by a combination of fermentation and chemical extraction, and the characterization of vulcanizates are described.

CRYPTOSTEGIA, a leafy tropical vine native to Madagascar, produces a latex which contains rubber. This rubber has been marketed from time to time; its properties are generally similar to those of hevea rubber (*1*). The leaves of the plant contain benzene-soluble material considered to be rubber (*4*). Two species, *Cryptostegia grandiflora* and *C. madagascariensis*, were introduced into this hemisphere, and the former has become widely dispersed throughout the West Indies, Central America, Mexico, and the extreme southern portions of the United States. For a number of years the United States Department of Agriculture has conducted botanical investigations upon *Cryptostegia* as a possible source of rubber (*13*). This work confirmed the existence of rubber in the leaves. Jenkins reviewed the information available in January, 1943, on *Cryptostegia* as an emergency source of rubber (*5*).

Properties of the leaf rubber became of interest when plantations were established with the primary aim of producing latex rubber from cryptostegia stems, since large quantities of leaves would thus be made available from normal pruning operations. Moreover, it was possible that the plant would be grown for the production of leaf rubber alone, provided its quality was high and satisfactory means of recovery could be worked out. In fact, the labor required to tap the vines for latex practically precluded the employment of this process in the United States and Mexico.

The characterization and isolation of the leaf rubber have been of primary interest. Whittenberger, Brice, and Copley (*19*) demonstrated that only 10 to 15% of the rubber in the mature leaf occurs in the latex ducts; 85 to 90% is embedded in the individual chlorenchyma cells as discrete globules. This "cell rubber" is, of course, not directly available by tapping the latex system of the leaf and has not been satisfactorily recovered by pebble-milling procedures of the type used in the production of guayule rubber. The latex duct rubber of the leaves is essentially the same as the rubber obtained by tapping the latex system in other portions of the plant. In the dried leaves it is coagulated and therefore contains the non-rubber constituents of the latex, which are largely removed during coagulation of latex obtained by tapping procedures.

Extensive studies were carried out on fermentation as a step in the recovery of the leaf rubber. An anaerobic fermentation by *Clostridium roseum* was worked out in which more than 60% (dry weight) of the leaves is digested. After two days' incubation the leaves are well digested and disintegrated. This slurry is passed over a vibrating screen (80 × 80 meshes to the inch). The liquid and protoplasts pass through, and the bagasse (cuticle, veins, and small stems) remain on top. The latex ducts in cryptostegia leaves are closely associated with the veins and, owing to their length, are trapped in the bagasse. The bagasse is suspended in one half the original volume with water and again screened to recover an additional quantity of protoplasts that are trapped mechanically. The bagasse is freed of excess water by pressing and then dried. The protoplasts (specific gravity 1.17–1.27) are recovered from the liquor in which they are suspended by gravity settling and decantation; they yield a slurry containing about 4–7% solids. The slurry is further freed of soluble materials by rediluting with water, settling, and decanting. Figure 1 shows the fermentation equipment. The bacteriological aspects of this fermentation have been described elsewhere (*12*).

This paper presents two procedures for the recovery of the cell rubber from the fermentation products. The first consists in isolation of the protoplasts followed by a "caustic cook" to liberate the rubber. The photomicrographs of Figure 2 illustrate the successive stages in this process. By the second procedure all the leaf rubber is extracted by benzene. Chemical and physical data on the raw rubber obtained by these processes are given, and certain properties of the vulcanizates are evaluated.

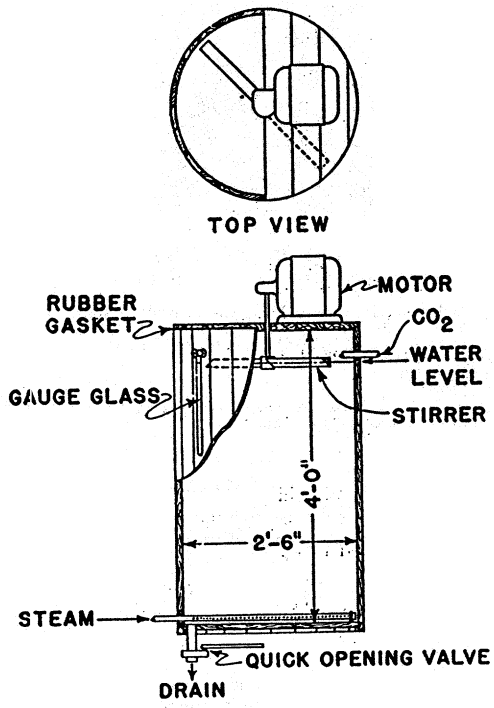

Figure 1. Anaerobic Fermentation Tank

CAUSTIC-CREAMING PROCESS

Previous work demonstrated that the protoplasts obtained by fermentation could be digested by dilute alkali and thus release the rubber globules (*12*). Boiling a 10% suspension of the protoplasts for 20 minutes in water containing 2% sodium hydroxide was the standard procedure in the work described here. One per cent powdered JZF (4,4'-diphenylphenylenediamine), based on the amount of rubber, was added as antioxidant. The suspension containing the liberated rubber globules formed a "cream" when it stood without stirring. The cream was siphoned off the surface, dis-

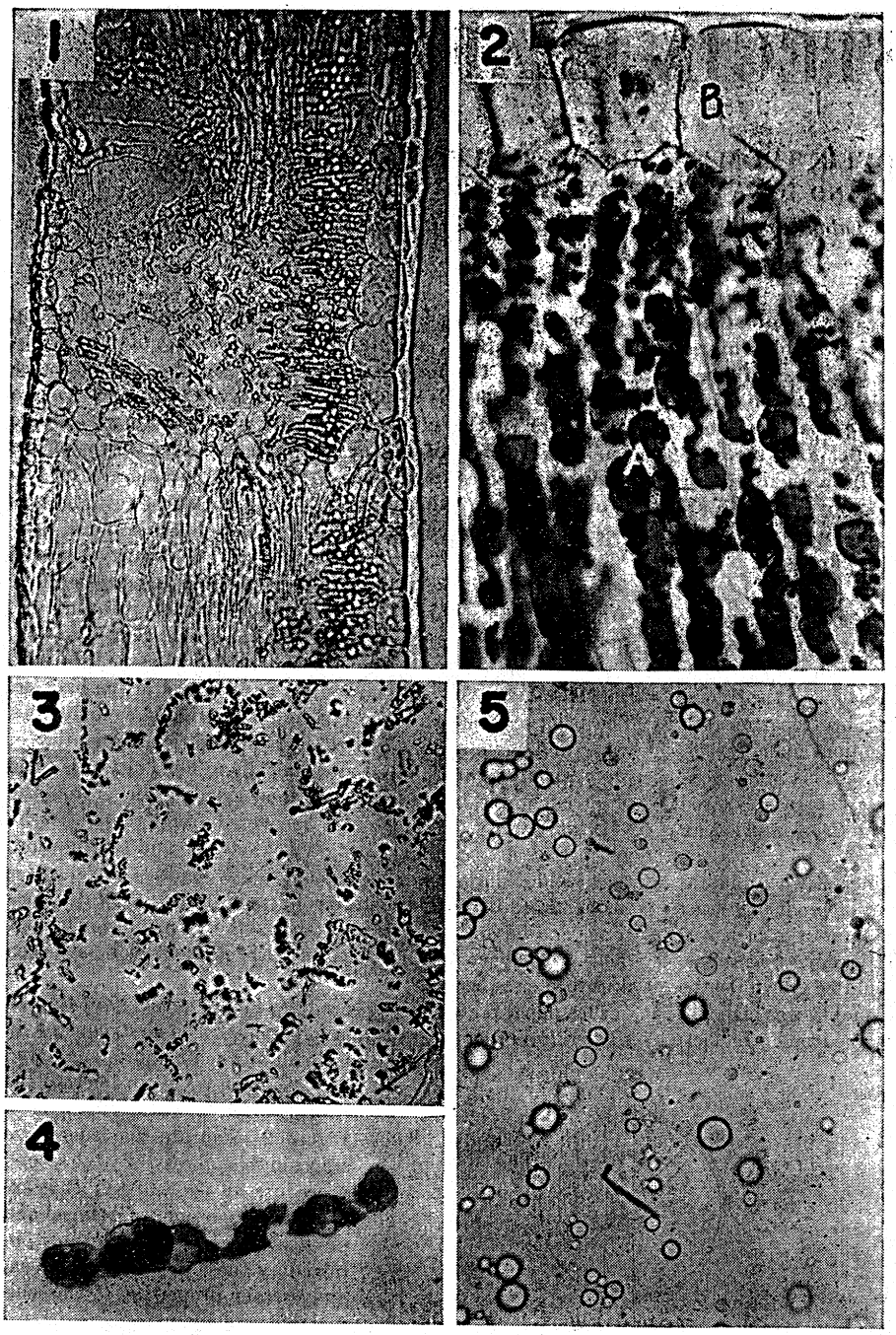

Figure 2. Photomicrographs of Material Prepared from an F_1 Hybrid of *C. madagascariensis* and *C. grandiflora*

1. Cross section of preserved specimen of mature leaf, unstained (×130). Rubber globules visible in chlorenchyma.
2. Cross section of fresh, living, senescent leaf (×460). *A*, rubber-bearing globules and chloroplasts within palisade cells; *B*, upper epidermis.
3. Protoplasts from retted leaves (×130).
4. Protoplast from retted leaf (×1000).
5. Suspension of rubber-bearing globules isolated from protoplasts by alkaline digestion of protoplasts (×460). Many of the globules are not in focus.

persed in water, and recreamed. After it had been washed in this manner several times, the pH was adjusted to 4.6 with acetic acid, whereupon the globules "clumped", the cream separated in less than half an hour, and clear serum remained. If the alkaline liquor was adjusted to pH 4.6 without prior creaming, a heavy precipitate of proteinaceous material trapped the rubber globules and carried them down with it.

CREAMING STUDIES. The distribution of particle size and density of the globules were determined to facilitate development of methods for their recovery. About 4 liters of the liquor produced by boiling the protoplasts in alkaline solution was allowed to cream overnight in a depth of approximately 25 cm. A small sample (0.1 ml.) of the cream was taken off and diluted for microscopic observation (sample A), after which the dispersion was stirred thoroughly with a mechanical stirrer, sampled (B), and subjected to centrifugation at 2000 r.p.m. (925 × gravity) for different periods. After centrifugation the rubber was completely removed from the surface and the liquor underneath analyzed for rubber (Table I). Microscopic observations were carried out upon the liquor of the 20-minute sample (C).

The analytical results reported in this paper were obtained by Willits and co-workers (*20*). The samples were extracted with benzene, benzene insolubles were determined by filtration of the benzene solution, and rubber hydrocarbon was then determined gravimetrically by precipitation as the tetrabromide. Resins were calculated by difference. Rubber could not be quantitatively extracted from the raw leaves unless a preliminary treatment with oxalic acid was applied. Resins and rubber were determined by a modified Spence-Caldwell procedure.

The samples were prepared for microscopic examination by mixing 0.01 ml. of each with 0.01 ml. glycerol on a slide, carefully evaporating the water, and covering with a cover slip. Brownian motion of the particles was thereby suppressed, and the globules could be accurately measured and counted. The number of globules in each size group was determined in a large number of fields selected at random; a calibrated eyepiece micrometer was used. (The count of the smallest globules in whole liquor B is an estimate obtained by calculation from the count of the centrifuged sample C. This was necessitated by the denseness of the slide of the whole liquor and the number of bacterial cells present, which caused difficulty in counting the smaller particles. An appreciable error in this estimate would not affect the weight distribution markedly, for the total weight of this fraction was only 4% of the whole.) The data are presented in Table II and Figure 3. Data for hevea latex taken from Lucas (*8*) are included in Figure 3 for comparison.

To calculate the results, the globules were assumed to be homogeneous and identical in composition, independently of size. This assumption is justified within the limits of accuracy desired here. Analysis of the data shows that 91% of the total number of globules in the whole liquor were present in the two smallest size classifications (1 and 2μ diameter), yet they constituted only 16% of the weight of the rubber present. These smaller particles were almost entirely absent in cream A which rose during 16 hours' standing. Moreover, they constituted all the globules in liquor C after a centrifugation equivalent to 300 hours' standing.

The specific gravity of the globules was determined by the equilibrium position they assumed when centrifuged in water-alcohol mixtures. The average specific gravity of the globules recovered by creaming was 0.92, although for some it was as high as 0.96. The specific gravity of the alkaline cook liquor was 1.02. Substitution of these values and the microscopically determined